Spatial and temporal regulation of G protein signalling cascades

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A vast array of cellular processes are controlled by guanine nucleotide-regulated molecular switches including hetero-trimeric G-proteins, for the transduction of signals across the plasma membrane and monomeric G-proteins, involved in cell proliferation and division. The activity of the G proteins within these signalling cascades requires tight regulation in a cycle of GTP-GDP exchange.

The spatial and temporal regulation of G protein signalling, through the action of GTPase activating proteins (GAPs) is known to be critical to maintain cells viability and aberrant signalling is the primary cause of many diseases; hyperactivated Ras proteins are involved with many human cancers[(1)]. The yeast pheromone-response pathway provides an attractive model organism to study G protein signalling: pheromone induces GPCR activation and the Gα subunit (Gpa1) activates a small, monomeric G protein, Ras1, subsequent morphological and transcriptional response can then be analysed using microscopy and reporter gene assays [(2)]. Previously we have shown the importance of the negative regulator of the Gα subunit, Rgs1 and its dual positive and negative role on the cells response [(3)]. Here we show that the negative regulator of Ras1, Gap1 is required for cells to survive high pheromone stimulation. Removal of Gap1 does not affect the ability of cells to grow and divide in unstimulated conditions (no significant difference in doubling time: p >0.05, n=6) However, when exposed to high concentrations of pheromone, these cells display abnormal morphologies, increased deposits of cell wall material within the cytosol and significantly reduced viability; in a population of cells lacking Gap1, 88 ± 1.2% fail to survive pheromone treatment compared to 10 ± 2.2% (n=5 ± SEM) of wild type cells. These defects can be partially rescued through the addition of the essential Ras1 effector, Cdc42 with 50 ± 1.5% (n=5 ± SEM) of cells now able to survive pheromone treatment. Taken together these data indicate that cells containing G proteins with a defect in their ability to hydrolyse GTP become ‘stuck’ in an activated state, requiring the action of GAPs to release the G protein from its effectors. We conclude that, in the absence of Gap1, Cdc42 becomes sequestered within a Ras1-GTP complex and can no longer perform other, essential roles. Our results highlight the importance of desensitization of G protein signalling, as downstream components of the signalling cascade are also essential for cells ability to grow and divide under normal conditions.

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2. Didmon, M. *et al* (2002) *Curr. Genet.* **41**:241-253

3. Smith, B. *et al* (2009) *Cell Signal.* **21**:1151-1160